IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicants:	Glenn McGall and Lisa Kajisa	
Application No.:	09/810,434	Group: 1635
Filed:	March 15, 2001	Examiner: Epps, Janet L.
Confirmation No.:	6484	
For:	Phosphate Ester Oxidation in Nucleic Acid Array Preparation	

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, P.O. Box 2327, Arlington, VA 22202

on 3/10/03 (Date) Denise Candelo (Signature)

Denise Candelo

Typed or printed name of person signing certificate

DECLARATION OF GLENN McGALL UNDER 37 C.F.R. 1.132

Assistant Commissioner of Patents and Trademarks
P.O. Box 2327
Arlington, VA 22202

Sir:

I, Glenn McGall, Ph.D., of Mountain View, CA, declare and state that:

1. I am an inventor with Lisa Kajisa of the subject matter described in U.S. Application No. 09/810,434. This patent application was filed on behalf of Affymetrix, Inc., 3380 Central Expressway, Santa Clara, California, 95051.

2. I have thoroughly studied the above-identified application and the Office Actions mailed from the Patent Office on July 3, 2002 and October 29, 2002. The Examiner states that Claims 1 to 17 are rejected as being unpatentable over Earhart *et al.* (U.S. Patent No. 6,300,137) in view of McGall *et al.* (U.S. Patent No. 6,147,205). The Examiner states that it is unclear whether the unexpected results observed with 0.02 M iodine, which are described in the specification, will also be observed with concentrations of from about 0.005 M to about 0.05 M iodine. The Examiner states that it is necessary to show that the results of phosphite ester oxidation with the particular claimed concentration range of iodine are unexpected compared to phosphite ester oxidation with a concentration of about 0.1 M iodine.

3. I have performed extensive research related to the preparation of nucleic acid arrays, which typically require the step of oxidizing a phosphite ester with iodine. The results of my work are disclosed in numerous patents. Examples of such patents include, for example, U.S. Patent Nos. 5,599,695, 5,831,070, 5,959,098, 6,150,147, 6,239,273 and 6,307,042.

4. The ability of nucleic acids to serve as an analytical tool is affected by the purity of the nucleic acid sequence that is synthesized. An ideal synthetic method will efficiently couple nucleotides to a nascent nucleic acid polymer while not otherwise chemically modifying the base or the sugar moieties. This ensures that the nucleic acid polymer has the intended composition.

We have unexpectedly found that lowering the concentration of iodine used in one of the final steps of nucleic acid array preparation, namely oxidizing phosphite esters to phosphate esters, improves the functional performance of the nucleic acid arrays. Previously, it was thought that functional performance of a nucleic acid array would not be compromised when the iodine concentration was sufficient to completely oxidize all phosphite esters to phosphate esters, where an iodine concentration of 0.1 M or greater is standard. However, we have found that the functional performance of nucleic acid arrays is diminished when they are prepared using an iodine concentration of 0.1 M, presumably because of excessive oxidation of the nucleic acid molecules.

5. The functional performance of nucleic acid arrays can be measured by the fluorescence

intensity of a nucleic acid array that is hybridized with a target sequence. We performed a series of experiments to confirm the range of concentrations that are effective in decreasing unwanted oxidation of the nucleic acids, thereby increasing the fluorescence intensity produced by the array in a hybridization assay.

The experiments used the following materials:

Substrates:	Fused silica, with Single Bis Silanation
Mask Set:	Human Gene FL
Amidites:	5'-MeNPOC PAC deoxyadenosine B-cyanoethyl phosphoramidite
(all Amersham	5'-MeNPOC iso-butyl deoxycytidine, B-cyanoethyl phosphoramidite
Pharmacia)	5'-MeNPOC iso-propyl-PAC deoxyguanosine B-cyanoethyl phosphoramidite
	5'-MeNPOC thymidine B-cyanoethyl phosphoramidite
Linker:	MeNPOC PEG Linker Phosphoramidite (Amersham Pharmacia)
Activator:	4,5-dicyanoimidazole, 0.25 M in acetonitrile (Glen Research)
Solvents:	Acetonitrile (Burdick and Jackson)
	CAP A (10% acetic anhydride, 10% lutidine, 80% tetrahydrofuran; Glen Research)
	CAP B (16% 1-methylimidazole, 84% tetrahydrofuran; Glen Research)
Oxidants:	0.01 M - 0.1 M iodine, 10% water, 20% pyridine, balance tetrahydrofuran
	Additional oxidant formulations prepared internally

Synthesis of the arrays was conducted as described in "High-Density GeneChip Oligonucleotide Probe Arrays", Glenn H. McGall and Fred C. Christian, *Advances in Biochemical Engineering/Biotechnology* 77: 21-42 (2002), attached as "Exhibit A", with the exception that the iodine concentration in the oxidant solutions varied as shown below in Experiments 1 and 2. In addition to iodine, the oxidant solutions contained 10% water and 20% pyridine, and the balance was tetrahydrofuran. Variations in reagent composition were evaluated

using a standard Affymetrix product array known as the Human Full-Length array. This array contains several hundred thousand probe sequences complementary to different regions of some 20,000 human gene sequences. For quality control (QC) purposes, this array also contains a number of control probe sequences which are designed to detect bacterial gene transcripts and synthetic oligonucleotide targets. The latter are "spiked" into test samples in order to assess the performance of the array with respect to its ability to detect targets in a test sample at known concentrations.

A number of quality assurance measures were employed in the synthesis of the arrays. The chemical delivery system was checked to ensure that delivery volumes, pressure checks, and flow rates were within manufacturing specifications. Each set of experiments used the same manufacturing lot numbers for each chemical to eliminate lot-to-lot variations. Standard "control" arrays were synthesized for each set of experiments conducted. To minimize possible environmental variables, dual runs were performed (control and experimental) for side-by side comparisons.

Following synthesis of the arrays, each set of control and experimental wafers were processed in parallel to minimize variations outside of the synthesis process. The post-synthesis processing included chemically deprotecting all of the wafers, then sawing and packaging the arrays for hybridization studies, concurrently. To assess the functional performance of the arrays, the control and experimental wafers from the same set of experiments were hybridized and scanned concurrently. Reference arrays were used to monitor day-to-day assay variation.

To illustrate, the results for several of the hybridization experiments are shown below. In each of these experiments, wafers with the human full length array and the control arrays described above were probed with equal concentrations of one or more control target sequences complementary to those on the wafer. The target sequences each contain a fluorescent marker, so that the amount of the target sequence bound to the array can be assayed. In Experiment 1, the reported fluorescence intensity is that for the feature containing the complement to the Control Block 3 sequence, one of the synthetic oligonucleotides added for QC purposes. In Experiment 2, the reported fluorescence intensity represents the average intensity of the signal from about 20 probe sequences on the wafer that are complementary to different portions of the Bio-C gene

transcript – one of the bacterial gene transcripts added for QC purposes. A greater reported fluorescence intensity indicates a higher detectable signal due to binding of the control target sequence to the array.

Experiment 1: Human Full Length Array with Control Block 3 Target Sequence

Iodine Concentration	Average Hybridization Signal Intensity
0.01 M	12542
0.02 M	16330
0.05 M	12683
0.10 M	10159

Experiment 2: Human Full Length Array with Bio-C Target Sequences

Iodine Concentration	Average Hybridization Signal Intensity
0.01 M	3464
0.02 M	4739
0.05 M	3489
0.10 M	2754

6. Based on the increase in fluorescence intensity of the wafers in these hybridization studies, we have concluded that using a lower concentration of iodine in nucleic acid array preparation results in an array with improved functional performance. Overall, the data presented above demonstrate that improved functional performance is observed for iodine concentrations ranging from 0.01 M to 0.05 M, as compared with the 0.1 M or greater concentrations of iodine used in the prior art.

These results are presented here for illustrative purposes, but are representative of a large volume of data that has been generated as part of an extensive optimization of the oxidant reagent formulation.

7. I hereby acknowledge that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Glenn McGall

Glenn McGall

03/06/03

Date